

ON THE GERM THEORY OF PUTREFACTION AND OTHER FERMENTATIVE CHANGES.*

AFTER some introductory remarks referring to the various other theories which had been entertained on this subject, viz., the oxygen theory, the theory of spontaneous generation, and that of chemical ferments, the author stated that the researches of Pasteur had long since made him a convert to the germ theory, which attributes the alteration experienced by exposed organic substances to the development within them of minute organisms springing, like larger living beings, from parents like themselves; and that this belief had been since continually strengthened by the results of the antiseptic system of treatment in surgery, which he had founded on that theory as a basis.

But his attention had been afresh directed to the subject about a year and a half ago by a remarkable paper by Dr. Burdon-Sanderson,† in which experiments were recorded, leading to the conclusion that Bacteria, unlike the spores of fungi, are deprived of vitality by mere desiccation at a moderate temperature, so that while a drop of water from ordinary sources or the contact of a moist surface is sure to lead to Bacteric development and consequent putrefaction in an organic substance susceptible of that change, the access of dust from exposure to the atmosphere induces merely the growth of fungi and comparatively insignificant chemical alteration.

If this were true it would be needless to provide an antiseptic atmosphere in carrying out the antiseptic system of treatment; and all that would be requisite in the performance of a surgical operation would be to have the skin of the part about to be operated on treated once for all with an efficient antiseptic, while the hands of the surgeon and his assistants and also the instruments were similarly purified; a dressing being afterwards used to guard against the subsequent access of septic material. Thus the use of the spray might be dispensed with, and no one would rejoice more than himself in getting rid of that complication.

Such being the practical importance of the conclusion referred to, he determined to subject it to a searching experimental test:

The material first employed was urine, not boiled, as it had commonly been in previous investigations, but obtained, by a very simple antiseptic process, perfectly uncontaminated in its natural condition, in which it proved a far more favourable nidus for the development of organisms than in the boiled state, as indeed might have been anticipated, since it contains unaltered the complicated organic substance termed the mucus, which has been sometimes regarded as a chemical ferment of urine. Nevertheless, when a wine-glass, together with a small porcelain evaporating dish, to serve as a cover, had been heated, like the vessels used by Dr. Sanderson, far above the boiling-point of water, and allowed to cool (a process conveniently designated by the term "heated"), and afterwards charged with the unboiled urine, and placed under a glass shade as an additional protection against dust, it was found that the fluid remained free from organic development or putrefactive change for months, till at last it dried up into a saline mass. On the other hand, if a glass so charged was exposed to the air by removing the shade and cover for a while, organisms appeared in it of various kinds, and among the rest, in several instances, Bacteria. Thus it was shown on the one hand that Bacteria might arise from atmospheric exposure, and on the other hand that a porcelain cover and glass shade afforded absolute security against the introduction of organisms from without. If, therefore, the exposure of such a glass for a limited period chanced to lead to the introduction of any one organism unmixed with others, the opportunity was afforded of studying the behaviour of that organism, either in the same medium for a protracted period or in other media in similar glasses, inoculated by means of a heated pipette or glass rod. For it was found as a matter of experience that exposure for the few seconds or fractions of a second necessary for performing the inoculation or withdrawing a little fluid for examination did not involve any considerable risk of accidental contamination.

Early in the investigation it was ascertained that the putrefaction of urine might take place without the occurrence of Bacteria, in presence of minute granules in irregular groups, in

such numbers as to make the liquid milky; their organic nature being clearly proved by fissiparous generation observed to take place in them, though in a different manner from that which is seen in Bacteria. To this form of organism the name "Granuligera" has been provisionally applied.

In one of the experiments related, two drops of water from the tap having been added to a glass of Pasteur's solution, the result was not in the first instance the general opalescence due to Bacteria in a liquid, but a deposit which proved to be a minute filamentous fungus producing abundant spores (conidia) on its branches. These spores after separation often produced young plants like their parents; but there were also seen in abundance precisely similar spores multiplying by pullulation like a Torula (to retain the old use of the name as applicable to organisms like the yeast plant).‡ And there were also present multitudes of more slender filaments which were seen to break up into Bacteria, while in several instances these filaments were observed springing from spores undistinguishable from those of the fungus.

The view that some filamentous fungi may give origin to both toruloid and Bacteric forms was soon afterwards confirmed by another experiment. § A "heated" wine-glass was taken into the open air during a drizzling rain, and the cover being lifted, some rain-drops were allowed to fall into it, after which uncontaminated urine was introduced. The result was the production of a pullulating delicate Torula, totally different from the yeast plant; forming a granular deposit on the sides of the glass, and an abundant scum, both in the urine and also in Pasteur's solution on repeated inoculations. Portions of both liquids containing this organism having been set aside under circumstances permitting only very slow evaporation, they were examined again eight months later, when a delicate filamentous fungus was found in both, bearing conidia resembling the cells of the Torula, while similar spores were seen multiplying by pullulation, and some of the buds were in a slender form undistinguishable in character from the Bacteria which in the case of the urine were observed swimming in the liquid.

An organism which in the first instance was observed for weeks together growing as a mere Torula having thus, as it appeared, developed into a filamentous fungus, after remaining for months in the same solution, hopes were excited that a corresponding observation might be made with regard to the yeast-plant, and this led to a careful examination of a low white mould, referable to the genus *Oidium*, which was observed in a glass of Pasteur's solution to which yeast had been added several weeks previously. The hope was disappointed, but some interesting facts were elicited: for the fungus was found to vary remarkably according to the quality of the medium in which it grew, having sometimes the aspect of an *Oidium* with fructifying filaments, sometimes a purely filamentous structure, sometimes a loosely-jointed growth producing abundant oval spores destitute of nuclei, and often pullulating like a Torula, and lastly a purely toruloid form of an entirely different aspect, composed of spherical nucleated cells, occurring in urine, and operating as a powerful putrefactive ferment upon that fluid. Yet totally dissimilar as the different forms of this fungus might appear, their identity was demonstrated by observing with the microscope the actual growth of one from another when transferred to a new medium on a slide of thick glass excavated round a central island, so as to provide a sufficient supply of oxygen to last the growing fungus for a long period. The slide and its thin covering glass were heated between metallic plates to diffuse the heat and prevent cracking of the glass, so arranged as to guard against the entrance of dust during cooling, and all instruments, such as forceps and needles, employed in the subsequent manipulations, were "heated" before being used, the thin covering glass being luted down with melted paraffin applied with a hot steel pen. "Glass gardens" of this construction stocked with various organisms in various media proved extremely useful means of investigation. Samples of the organism introduced were sketched with camera lucida immediately after introduction, and their subsequent development observed with perfect precision. In this way, in the case of the *Oidium*, spherical nucleated cells of

* The toruloid pullulation of spores of some minute filamentous fungi had been previously observed by De Bary. See "Morphologie und Physiologie der Pilze," &c., von Dr. A. de Bary, p. 183.

† This view has been expressed by various other authors, but has been hitherto incapable of demonstration in consequence of the uncertainty whether things which seem to grow from one organism may not be merely the result of the accidental presence of others.

* Abstract of a communication made to the Royal Society of Edinburgh, April 7, 1873, by Prof. Joseph Lister, F.R.S.

† See 13th Report of the Medical Officer of the Privy Council.

the toruloid form of the organism were observed to sprout into beautiful filamentous fungi, and these again, as the fluid became vitiated by the growing fungus, were found to reproduce as conidia the spherical toruloid cells.

Among other media inoculated with this *Oidium* was a solution of albumen obtained by treating a fresh-laid egg with a solution of carbolic acid, to destroy any organisms adhering to the shell, and then breaking it with carbolised fingers into a "heated" vessel containing water that had been boiled and allowed to cool protected from dust, the solution being afterwards cleared by passing it through a boiled filter in a "heated" funnel with "heated" cover. This fluid had remained during the half-year which had since elapsed, free from putrefaction or any other change, except where organisms had been introduced, although the air had free access to it; * a fact which indicates pretty clearly that the putrefaction of eggs, which has been regarded as a stumbling-block in the way of the germ theory, must somehow or other be brought about by the penetration of ferments through the shell and membrane. This, indeed, becomes intelligible enough if we admit that Bacteria may be formed from fungi, and remember how the filaments of some parasitic fungi perforate the epidermis of leaves.† In a glass of this albuminous fluid the *Oidium* grew very slowly and feebly, but its development was accompanied by a remarkable alteration in the liquid, which, in the course of six weeks, changed from the colourless purity of spring water to the dark brown, almost black, appearance of porter. Yet the dark brown liquid remained perfectly free from smell, proving, what the author had long suspected as the result of experience in antiseptic surgery, that an albuminous fluid may undergo fermentation with odourless products.

Another experiment given in full detail was performed with milk upon the same principle as those with urine and albumen, in the hope of removing another stumbling-block in the path of the germ theory. For, according to the high authority of Pasteur, milk forms an exception to organic liquids in general, in the circumstance that a greater elevation of temperature than the boiling-point of water is required to kill Bacteria contained in it.‡ But the advocates of the theory of spontaneous generation reply that any Bacteria present would be certainly killed by boiling, and therefore the subsequent appearance of living Bacteria in the boiled milk in Pasteur's experiments is proof of their spontaneous evolution from the chemical constituents of the liquid. If, however, by the use of antiseptic means, milk could be obtained uncontaminated from the cow, there being no organisms to kill, boiling might be dispensed with, and the milk, like the unboiled urine, should remain free from organic development or fermentative change, if kept protected in "heated" vessels. Accordingly, five flasks with glass caps, and six test-tubes with wider test-tubes to cover them, having been heated, and allowed to cool under glass shades in the stable where the experiment was performed, the udder and adjacent skin of a cow were well washed with a strong watery solution of carbolic acid, which was also applied with a small syringe to the outlets of the milk ducts, the teat being held in the finger and thumb to prevent the entrance of the solution into the udder, and a milkman with his sleeves tucked up, and his hands and arms washed with the anti-septic lotion, was directed to milk into the glasses as their covers were successively raised. The cow did not give milk at all freely, and a considerable time was occupied in charging the flasks, but the small quantity required for each test-tube was got by a single squirt from the teat, with almost momentary exposure. Yet not only in all the flasks but in all but one of the test-tubes organisms made their appearance. In one of the test-tubes, however, the unboiled milk had hitherto (for a quarter of a year) remained entirely unaltered. One such success was as clear evidence against the hypothesis of spontaneous evolution of organisms as if all the glasses had remained free from them, and their occurrence in the other ten proved a most fortunate circumstance. For no two of them were alike in the organisms they contained, and in several instances there was apparently only some one species unmixed with others, so that the opportunity was afforded of studying various different organisms as modified by other media, and as regards any fermentative influence which they might exert upon those media. Among the organisms in the milk glasses were Bacteria of different species, to judge from their size and other appearances, as well

* Dr. Burdon-Sanderson had previously preserved unboiled white of egg unchanged for six months in a "heated" tube containing air, hermetically sealed.

† See De Bary, op. cit., page 216.

‡ See *Annales de Chimie et de Physique*, 1862, p. 60.

as numerous kinds of fungi; and when they were introduced into a series of glasses of the albuminous liquid before described, it was found that while some of the fungi grew in it others refused to do so, and while Bacteria obtained by adding a drop of water to urine thrived in the albuminous fluid, not one of four inoculations of Bacteria from four milk glasses was followed by any result. Thus was afforded, it is believed, for the first time, distinct physiological proof of real differences among Bacteria. But what was still more unexpected was the fact that when the inoculation was practised in a series of glasses of urine, two of the Bacteria refused to grow even in that liquid, which had been previously regarded as a peculiarly favourable nidus for Bacteria development. This fact, besides serving still more clearly to differentiate the various species of Bacteria, suggested a possible explanation of the failure of experiments with milk in the hands of others. For if organisms thrive in milk which cannot grow in urine at all, milk must be a more difficult fluid to work with in experiments which aim at excluding organic development. Hence it seemed worth while to try again the effect of boiling milk, but in doing so to adopt more rigorous precautions against the entrance of organic germs. There could be little doubt that the organisms which appeared in the various milk glasses of the experiment above related entered during the cooling, which though it took place just as in the successful experiments with urine, led to failure in the case of the milk, partly from the favourable nature of that liquid for organic development, and partly no doubt from the atmosphere of the stable being much more loaded with organic germs than that of the author's study. The new precautions adopted were in the main these. The small wine glasses (liqueur glasses) into which the fluid was to be decanted were covered, together with their glass caps, while still very hot, with cotton wool secured by fine iron wire tied tightly round below the cap, so as effectually to filter the air that entered during cooling; after which the cotton was carefully removed and the glass placed under a small glass shade on a separate piece of plate glass. For heating the flask in which the milk was to be boiled a very high temperature was requisite to ensure destruction of all life in the considerable volume of air which it contained; and this was arranged for by binding asbestos with wire round the junction of the neck of the flask and the glass cap, and then roasting the flask over a large Bunsen's burner. The asbestos, which proved as good a filter as cotton wool, was removed after cooling, and the cap being lifted, a long "heated" funnel was passed quickly into the flask and the milk poured in through it after wrapping a piece of carbolised rag round the funnel and neck of the flask to exclude septic dust: scrupulous care being taken to avoid touching the neck of the flask with the moist end of the funnel as it was withdrawn. By this means security was obtained against the presence of any living organism inside the flask except in the fluid at the bottom of the vessel. The cap was then re-applied and carbolised cotton wool tied over it to filter regurgitant air during the boiling. The necessity for the air-filter was made very manifest during ebullition from the great tendency of milk to froth, involving the necessity of frequently removing the flame, fresh air entering on every such occasion: another peculiarity of milk which served further to explain the failure of previous experiments. But the efficiency of the means employed was shown by the appearance of the flask as exhibited to the Society. For although seven weeks had passed since it was filled, the milk was seen to be perfectly fluid and with no appearance of alteration.

All trouble occasioned by frothing, involving constant watching to prevent the froth from wetting the cotton, was afterwards avoided by acting on the suggestion of Mr. Godlee, of University College, London, who happened to be assisting the author at the time, and immersing the flask in boiling water above the level of the liquid, instead of applying the flame directly. This method had the further advantage of avoiding any risk of "burning" the milk, and also any loss by evaporation. A second flask "heated" and charged with milk like the other and similarly covered with cotton wool, was kept in this way at 212° F. for an hour, and, after cooling, its contents were decanted off into twelve "heated" liqueur glasses, and in these it had remained during the seven weeks that had since passed perfectly free from change except when organisms had been intentionally introduced. To illustrate this the author drank, before the Society, the contents of one of the uninoculated glasses, which proved perfectly sweet and good.

It was a curious circumstance that on the morning following

the night on which the liqueur glasses were thus charged with boiled milk, the author received from Dr. Roberts, of Manchester, a copy of his paper describing how he had got over all the difficulties, as regards milk, by a different and very simple method.* But beautiful as Dr. Roberts's method was, and perfectly conclusive against the theory of spontaneous evolution, it would not have answered the author's purpose, as it was essential for his investigations that the liquid should be decanted from the flask into the liqueur-glasses. The decanting was effected by means of a "heated" syphon, with special precautions against the entrance of living organisms, as was fully explained to the Society.

The same plan of "heating" the vessels and decanting was afterwards followed with turnip infusion and with urine; and in proof of the security of the method, flasks containing the residual stock of these fluids after decanting into twelve glasses from each nearly six weeks before, were shown to the Society quite unchanged. And as further evidence of the trustworthiness of the system pursued, it was mentioned that out of six series of wine-glasses with about twelve in each series, containing albuminous fluid, urine (in two series), Pasteur's solution, boiled milk and turnip infusion, although portions of the contents had been often removed for investigation or inoculation, only two instances were known to have occurred in which any organism (a filamentous fungus) had made its appearance which had not been arranged for either by inoculation or prolonged exposure.

(To be continued.)

SCIENTIFIC SERIALS

Ocean Highways for July is a very interesting number. The first article, on the "Voyage of the *Polaris*," accompanied by six small maps, shows that notwithstanding the disastrous results of Captain Hall's venture, it proves more strongly than ever that a well-equipped Arctic expedition, taking the route of Smith's Sound, would be attended with results of the highest value. "In the present day," the writer concludes, "when the true methods of exploring are well known, and men of science have clearly enumerated the important problems that will be solved, and the numerous valuable results that will be derived from the labours of an Arctic Expedition, the reasons for despatching one have acquired tenfold force." This is followed by a long and extremely valuable and interesting account of "Personal Experiences of Venomous Reptiles and Insects in South America," by Mr. Richard Spruce, who has spent fifteen years in Equatorial Africa for the purpose of investigating the natural history of that region. The author's account of his experiences gives a vivid idea of the many dangers and trials to which devotees of science are exposed, in their endeavours to add to the sum of human knowledge. We would strongly recommend Mr. Spruce's interesting article to all who take an interest in the subject, on which, our readers may remember, there was recently some correspondence in *NATURE*. H. H. Giglioli contributes two very valuable letters from Dr. Beccari on his explorations in Papuasia, which are likely to be attended with very important results. Other papers in this number are "On Settlements on the Gold Coast," with a map; a paper on Khiva, by Rev. G. P. Badger, consisting of a catena of extracts from several eminent Arabic writers; the "Foot-paths of London," a sort of popular geological lecture, by Mr. H. P. Malet; and the second part of Prof. H. Mohn's article on the Meteorological Institute of Norway.

Bulletin de la Société de Géographie, May. The first article in this journal is by M. Charles Maunoir, on the work of the French Geographical Society, and the Progress of the Geographical Sciences during the year 1872.—Mr. W. Huber contributes an interesting paper on the telegraphic network of the globe, with a map showing at a glance how much has already been done in this way to annihilate distance, and how much remains to be done to complete this important work.—This is followed by the conclusion of M. Balansa's paper on New Caledonia, the present instalment treating specially of the Loyalty Islands.—M. Edouard Sayons gives an abstract of the contents of M. Hunfalvy's very interesting work on the Finnish Provinces of the Baltic; the work is published in Hungarian, and is an account of the author's explorations in the districts mentioned in the year 1870.

* See *NATURE*, Feb. 26, 1873.

SOCIETIES AND ACADEMIES

Royal Society, June 19.—"On a newly discovered extinct Mammal from Patagonia (*Homalodotherium Cunninghami*)," by William Henry Flower, F.R.S., Hunterian Professor of Comparative Anatomy, and Conservator of the Museum of the Royal College of Surgeons.

The author describes the complete adult dentition of a new genus of Mammal, founded on remains discovered by Dr. Robert O. Cunningham in deposits of uncertain age, on the banks of the River Gallejos, South Patagonia. The animal appears to have possessed the complete typical number of teeth, i.e. twenty-two above and below, arranged in an unbroken series, and of nearly even height, and presenting a remarkable gradual transition in characters in both jaws, from the first incisor to the last molar. The molars more clearly resemble those of the genus *Rhinoceros* than any other known mammal; and, judging by the general characters of the teeth alone, the animal would appear to have been a very generalised type of Perissodactyle Ungulate, allied through *Hyracodon* (a North-American Miocene form) to *Rhinoceros*, also more remotely to *Macrauchenia*, and, though still more remotely, to the aberrant *Nesodon* and *Taxodon*. The generic name *Homalodotherium* was suggested for this form by Prof. Huxley in his Presidential Address to the Geological Society in 1870.

"The Diurnal Variations of the Wind and Barometric Pressure at Bombay," by F. Chambers. Communicated by Charles Chambers, F.R.S., Director of the Colaba Observatory, Bombay.

The object of this paper is to bring to notice a remarkable relation that has been found to exist between the diurnal variations of the wind and the barometer at Bombay.

The observations made use of are the records of a Robinson's anemograph during the first three years of its performance, viz. from June 1867 to May 1870, and the corresponding hourly observations of the barometer and the dry- and wet-bulb thermometer, made at the Government Observatory, Bombay.

The mean results for each hour of the day during the whole period, and the mean diurnal relations of each element are tabulated and graphically represented by figures. The diurnal variation of the wind is then investigated, the most influential part of which is attributed to the land- and sea-breezes which blow from E.S.E. and W.N.W., and are shown to follow mainly the same law of progression as the temperature of the air, thus affording confirmatory evidence of the truth of Hadley's theory of the trade-winds as applied to land- and sea-breezes.

Some peculiarities of the curve representing the land- and sea-breezes are then pointed out, and these the writer concludes are due to the superposition of another distinct variation having two maxima and two minima in the twenty-four hours like the barometer variation; and he supports his views by a reference to the variation of the east components of the wind in the months of July and August, when the land- and sea-breezes have almost disappeared. This is found to exhibit a decided double period. The north components of the land- and sea-breezes are then approximately eliminated from the north components of the whole variation, and the variation which then remains exhibits a very decided double period in this direction also. These variations with double periods are regarded as indicative of the existence of a double diurnal variation in the general movements of the atmosphere. Upon this hypothesis typical diurnal variations of the wind are deduced for north and south low latitudes; that for north latitudes exhibiting a double diurnal right-handed rotation, and that for south latitudes a double diurnal left-handed rotation, and from these the diurnal variation of the barometer is deduced.

The movements of the wind-vane at Bombay are then analysed, and the writer concludes that the greater part of the excess of "direct" over "retrograde" rotation of the vane at Bombay is due to the diurnal variation of the wind.

Extracts are given from observations made at St. Helena, Toronto, and Falmouth, showing the character of the diurnal wind-variations at those places, and their greater or less agreement with the deduced typical curves. The writer maintains that these variations afford independently a possible, if not a probable explanation of that movement of the air which Dove had called the "Law of Gyration;" and in conclusion he points to the extent of their applicability in deducing weather probabilities, and to the method of discussing storms.